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TITLE: A STUDY OF BACTERIAL PLUGGING OF "THIEF ZONES" DURING OIL
FIELD DRILLING

FINAL REPORT

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EXECUTIVE SUMMARY

An initial study was performed which demonstrated that a *Bacillus* species, isolated from the Salton Sea in California, grew well and produced excessive amounts of polymer in loose sand and gravel and in the presence of drilling mud. Six cylindrical core experiments later showed that the *Bacillus* species and a *Pseudomonas* species isolated from a produced water source in Harmattan Alberta, also grew well and produced polymer in packed sand and gravel cores and in the presence of drilling mud.

Permeabilities of the cores were reduced to > 99% of their original in all cases, except when bacteria were absent. The vegetative *Bacillus* and *Pseudomonas* cultures plugged the cores in the shortest period of time. Plugging profiles of the *Bacillus* spores were similar except that the richer the nutrient the sooner they plugged. Several pore volumes of the Polymer Medium 2 nutrient were required to pass through the core before it plugged as compared to the cores that were injected with half-strength Brain Heart Infusion medium.

Analysis of the core sections for viable cell counts and carbohydrate production indicate high numbers of cells and polymer production. Colony counts and polymer production were the highest in the core containing the *Pseudomonas* injected with Sodium Citrate Medium. Cores containing the *Bacillus* spores had similar colony counts to each other but polymer production was significantly lower in the core without the drilling mud.

Scanning electron microscopy revealed that bacteria moved down the length of the core and developed a biofilm which was responsible for the decrease in core permeability.

INTRODUCTION

During drilling operations it has been observed that the drill bit often encounters high permeability layers and all of the drilling mud used is lost into these "thief zones". Sand and gravel alone does not plug these zones effectively so this study was designed to determine the organisms, nutrients and other growth parameters that would be suitable for use with sand and gravel to effectively plug the pores. It is believed that bacteria can provide fast and effective plugging of porous matrices when they are introduced into these pores and stimulated by nutrients to grow and produce exopolymers. In this study salt tolerant *Bacillus* spores or vegetative cells and *Pseudomonas* cells were injected into sand and gravel cores which either contained or did not contain drilling mud. These cores were injected with nutrients to promote growth and polymer production until the permeability of the core was reduced to > 99%.

TECHNICAL DISCUSSION

Milestone One

An initial experiment was designed to demonstrate bacterial growth and polymer production in loose sand and gravel and in the presence of drilling mud. In this experiment, a halophilic facultative anaerobe, isolated from the Salton Sea in California, was tested for growth and polymer production. Two milliliters of the *Bacillus* spores maintained in Chemically Defined Sporulating Medium (CDSM) (Appendix 1) were inoculated into a sterilized flask containing 85 mls of half-strength Brain Heart Infusion Medium (0.5 BHI) (Appendix 2), 5 grams of crushed heat treated berea sandstone core, and 10 mls of drilling mud. The drilling mud was obtained from Shell and was composed of 10 lb/bbl Bentonite, 5 lb/bbl Gypsum, 5 lb/bbl MyloGel (Starch), water, and NaOH (pH 9.0-9.5). All of the components used, except the spores, were autoclaved before they were added to the flask.

A second flask was prepared exactly the same way except that Polymer Medium 2 (PM-2) (Appendix 2) was used in place of 0.5 BHI. Polymer Medium 2 was used because it had a high salt concentration and was known to be an excellent medium for *Bacillus* cell growth and polymer production. The two flasks were placed in a shaker for the entire duration of the experiment which was conducted at 22°C.

Viable cell counts were performed at the beginning of the experiment and on days 2, 3, and 4 in order to determine whether

the cells remained viable and continued to grow in the presence of drilling mud. One milliliter of the spore, sand and nutrient medium mixture was taken from the flask to do serial dilutions in Phosphate Buffered Saline (PBS) (Appendix 3) dilution blanks. The dilutions were then spread plated onto 0.5 BHI agar plates and incubated for 48 h at $23 \pm 1^{\circ}\text{C}$ before the colony forming units (CFU) were counted.

Milestone Two and Three

A second experiment was started when it was confirmed that the bacteria were able to grow in the presence of drilling mud. Six cylindrical core experiments were set up using the core apparatus developed by Shaw et al., 1985 (2). A two cm^2 pad of six sterile gauze pieces was placed at the bottom of each core holder to prevent the core contents from falling through into the effluent. A five centimeter long rubber core holder with a hole in the center, 2 centimeters in diameter, was placed in the core apparatus on top of the gauze and filled with the sand, gravel and drilling mud mixture. Thirteen grams of sand and gravel for each core was prepared by crushing heat treated berea sandstone cores into small sand and larger gravel pieces. Three milliliters of 0.5 BHI broth was dripped onto the sand until it became slightly wet and then approximately 4 mls of drilling mud was added. All the components were mixed together and were poured into the core holder until it was level with the top of the holder. The whole unit was occasionally knocked against the countertop to settle the sand, gravel and drilling mud mixture and to expel any air bubbles

trapped inside. Permeability of the prepared core was estimated to be between 6 and 8 Darcies. Once the core was developed a pressure gauge was screwed onto the core and the core was ready for experimentation. Before assembling the the core, the core apparatus was sterilized for 4 h with ethylene oxide gas and the core components were autoclaved.

Depending on the core experiment, either one-half strength Brain Heart Infusion Medium (0.5 BHI), Sodium Citrate Medium (SCM) (Appendix 3), or Polymer Medium 2 (PM-2) was prepared and filter sterilized into sterile 4 liter thick walled erlenmeyer flasks containing a magnetic stir bar.

Various media and bacteria combinations were used for the different core experiments. Either 4 mls of the Bacillus culture or the Pseudomonas culture were used to inoculate the 4 liters of media. The media and bacteria combinations used were:

1. Bacillus spores followed by nutrient injection (0.5 BHI) without drilling mud.
2. Bacillus spores followed by nutrient injection (0.5 BHI) in the presence of drilling mud.
3. Bacillus spores followed by nutrient injection (PM-2) in the presence of drilling mud.
4. Vegetative Bacillus culture grown to exponential phase in 0.5 BHI in the presence of drilling mud.
5. Nutrient (PM-2) in the presence of drilling mud.
6. Pseudomonas culture grown to exponential phase in SCM in the presence of drilling mud.

One end of a 30 cm long tube (O.D. 9 mm and I.D. 6 mm) was attached and secured with a wire to the spout on the bottom of the Erlenmeyer flask while the other end was attached and secured with a wire to the top spout on the core apparatus. Bubbles from the core were squeezed out and shaken up through the tubing into the reservoir flask. A rubber stopper with two tubes running through it was placed in the mouth of the flask. One of the tubes was connected to a Nitrogen gas supply and the other was clamped shut and only used for a pressure release if needed. The nitrogen gas was turned on very slowly until the reading on the core pressure gauge was 3.5 psi. A constant pressure of 3.5 psi was maintained throughout the whole experiment.

Once the pressure was turned on the media moved through the core and out through the opening at the bottom of the core apparatus. An initial permeability reading (K_i) was obtained by using a stopwatch and a 10 ml calibrated cylinder to time how long it took for 10 mls of media to pass through the core. During the course of the experiment several other timed readings (K) were performed. The initial timed reading was considered to be 100% permeability and changes in core permeabilities were determined by dividing the flow rate through the core (K) by the initial flow rate (K_i).

Media containing the vegetative *Bacillus* and *Pseudomonas* was allowed to run continuously through the core. Cores containing the spores had media run through them intermittently until they

became vegetative cells and started to plug. For these cores the tube connecting the reservoir flask and the core holder would be clamped off between readings to prevent all of the media from running through. In all the cores once the permeabilities of the cores were reduced to $< 1\%$ the system was shut down.

During the experiment the fluid that passed through the core was collected in a calibrated cylinder to determine the total volume that had passed through the core. Effluent samples were also collected periodically to obtain the numbers of bacteria that passed through the core and out. A sample of the effluent was pipetted into a Phosphate Buffer Saline (PBS) blank to make a dilution. A dilution series was prepared and the solution from each tube was spread plated onto 0.5 BHI agar plates and incubated for 48 h at $23 \pm 1^\circ\text{C}$.

At the conclusion of the experiment the core apparatus was dismantled and the core was removed from the holder. The core was pushed up from the bottom and approximately seven 7 mm sections were cut off. Each section was further divided into subsections. One subsection was prepared for scanning electron microscopy (SEM) by dehydration and coating with gold-palladium (2). Another subsection was weighed and placed in a PBS blank and sonicated for 5 s in a sonic bath. Serial dilutions were performed and were spread plated onto 0.5 BHI agar plates and incubated for 48 h at $23 \pm 1^\circ\text{C}$. The other subsection was put into a tube containing 5 mls of concentrated H_2SO_4 for carbohydrate studies. Total carbohydrate assays were performed on the samples using the

procedure described by Dubois et al. (1).

RESULTS AND DISCUSSION

From both experiments, it was observed that both of the bacteria, *Bacillus* and *Pseudomonas*, grew well and produced polymer in the presence of drilling mud.

In the first experiment, using loose sand and gravel in a flask, the *Bacillus* spores continued to grow in the presence of drilling mud. They produced excessive amounts of polymer as seen by a thick matrix of growth attached to the top of the sand lying on the bottom of the flasks. Higher colony counts were obtained in 0.5 BHI than PM-2 (Figure 1) probably due to the fact that the 0.5 BHI is a richer nutrient media and the cells became vegetative cells quicker.

In the second experiment, in which sand and gravel cores were used, all of the cores, except when bacteria were absent, had a decrease in permeability $> 99\%$. Figure 2 shows the decrease in permeability of the cores against time. The vegetative *Bacillus* and *Pseudomonas* cores show the quickest decrease in permeability, then the *Bacillus* spores in 0.5 BHI and finally the *Bacillus* spores in PM-2. Vegetative cells are in an active stage and are larger so they would be expected to plug first. It took longer for the *Bacillus* spores in 0.5 BHI to plug but once they became vegetative cells they also plugged the core quickly. The *Bacillus* spores in PM-2 took the longest to plug because it took them longer to become adjusted to the high salt environment.

Three hundred milliliters of 0.5 BHI passed through the core

containing the vegetative *Bacillus* before the permeability was reduced to > 99% (Figure 3). The *Pseudomonas* which took the same amount of time to plug, required approximately 1500 mls of SCM to pass through the core before it plugged completely. If the *Pseudomonas* had been grown in the nutrient rich 0.5 BHI medium it would probably have had a similar plugging profile to the vegetative *Bacillus*. Approximately 500 mls of media passed through the core containing the *Bacillus* spores before they started to plug. The spores were injected into the cores and then liquid was run intermittently until a decrease in permeability was noted. Since there was medium running through the cores before a decrease in permeability was noted, it appeared that more media was required to plug the cores. In actual fact once the spores became vegetative cells very little media ran through the cores before they began to plug rapidly. The core containing the *Bacillus* spores in PM-2 media required the largest volume of media to pass through because it took longer for the cells to become vegetative and to initiate plugging.

Figure 4 and Table 1 displays the colony counts obtained from the sections of the various cores. The *Pseudomonas* core had the highest colony counts and the cores with the *Bacillus* spores had lower counts which were all approximately the same. The concentration of the start culture of the *Pseudomonas* was much higher than the start cultures of the *Bacillus* spores (Table 1) so the fact that the colony counts were higher was not unusual. Viable cells were found in every section of the cores so it appears

that in all of the cores the bacterial cells are able to move down throughout the length of the core.

The carbohydrate assay revealed that the core containing the *Pseudomonas* had the highest carbohydrate values (Table 2 and Figure 5). Polymer production of the vegetative *Bacillus* and the spores in 0.5 BHI and PM-2 in the presence of drilling mud were all lower and similar to each other. Polymer production was lowest in the core containing no drilling mud. Start culture concentrations and colony counts for this core were the same as the other spore cores so it could be assumed that the drilling mud had no significant effect on the viability of the cells but that it enhanced polymer production.

Scanning electron microscopy pictures were taken of the top, middle and bottom sections of two representative cores (Figures 6 and 7). SEM confirmed that the spores and cells were able to move all the way down through the core. Figure 8 shows what an uninoculated berea sand stone core looks like for comparison. *Pseudomonas* cells appeared to form a more confluent biofilm than the vegetative *Bacillus* cells (compare Figure 6 with Figure 7). Skin plugging occurred to some extent at the top of the cores but cells also moved down throughout the core and plugged.

CONCLUSIONS

It can be concluded that drilling mud enhances growth and polymer production of the bacteria that were used in this experiment. Depending on the situation, there are several bacteria and media combinations that can be used. Vegetative cells in a rich nutrient medium can be used if plugging is required immediately and spores can be used if plugging is required later in time. A rich nutrient medium such as 0.5 BHI can be used if polymer production is required quickly and more selective medias, such as SCM and PM-2, can be used if slower polymer production is required.

FIGURE LEGEND

Figure 1 Colony forming units / ml of the *Bacillus* spores in the shaker flasks containing 0.5 BHI ○ , and PM-2 ● .

Symbols for the cores used in Figures 2, 3, 4, and 5.

- △ *Bacillus* spores followed by nutrient injection (0.5 BHI) in the absence of drilling mud.
- *Bacillus* spores followed by nutrient injection (0.5 BHI) in the presence of drilling mud.
- ▽ *Bacillus* spores followed by nutrient injection (PM-2) in the presence of drilling mud.
- ◇ Vegetative *Bacillus* culture grown to exponential phase in 0.5 BHI in the presence of drilling mud.
- ⊙ Nutrient (PM-2) only in the presence of drilling mud.
- *Pseudomonas* culture grown to exponential phase in SCM in the presence of drilling mud.

Figure 2 Percent decrease in permeability (K/K_i) of the packed sand and gravel core against time (hr).

Figure 3 Percent decrease in permeability (K/K_i) of the packed sand and gravel core against volume (mls).

Figure 4 Colony forming units per gram (CFU/g) of each section in the cores.

Figure 5 Total carbohydrate assay showing the micrograms of polymer produced per gram of each section in the cores.

Figure 6 SEM shots showing *Pseudomonas* cells and polymer production at the top (A), middle (B), and bottom (C) of a packed sand and gravel core containing drilling mud. Bar indicates $5.0\mu\text{m}$.

Figure 7 SEM shots showing vegetative *Bacillus* cells and polymer production at the top (A), middle (B), and bottom (C) of a packed sand and gravel core containing drilling mud. Bar indicates $5.0\mu\text{m}$.

Figure 8 SEM shots of an uninoculated packed sand and gravel core containing drilling mud. Bar indicates $5.0\mu\text{m}$.

REFERENCES

1. Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28:350-356.
2. Shaw, J. C., B. Bramhill, N. C. Wardlaw, and J. W. Costerton. 1985. Bacterial fouling in a model core system. *Appl. Environ. Microbiol.* 49:693-701.

Table 1
Colony Counts
(CFU / gram)

Section of core	Bacillus Spores 0.5 BHI	Bacillus Spores 0.5 BHI (no drilling mud)	Bacillus Spores PM-2	Pseudomonas SCM
1	4.17×10^8	7.85×10^7	1.62×10^7	2.56×10^{10}
2	9.56×10^7	3.91×10^7	9.86×10^6	1.15×10^{10}
3	2.29×10^7	6.28×10^7	1.02×10^7	1.01×10^{10}
4	3.17×10^7	3.39×10^7	7.50×10^6	6.67×10^9
5	1.40×10^7	3.53×10^7	1.55×10^7	6.80×10^9
6	1.24×10^7	6.45×10^7	8.02×10^6	5.60×10^9
7	2.00×10^7	4.88×10^7	1.06×10^7	4.05×10^9

COLONY COUNTS FOR THE CORE CONTAINING VEGETATIVE BACILLUS
IN 0.5 BHI ARE NOT AVAILABLE.

START CULTURES:

Bacillus spores 0.5 BHI - 6.7×10^5

Bacillus spores 0.5 BHI (no drilling mud) - 1.23×10^5

Bacillus spores PM-2 - 6.5×10^4

Pseudomonas SCM - 5.5×10^8

Table 2

Total Carbohydrate Assay

(Micrograms / gram)

Section of core	Bacillus Spores 0.5 BHI	Bacillus Spores 0.5 BHI (no drilling mud)	Bacillus Spores PM-2	Vegetative Bacillus 0.5 BHI	Pseudomonas SCM
1	609.10	148.65	387.95	680.35	881.35
2	532.25	80.35	282.25	563.50	937.50
3	309.20	28.10	493.25	702.00	869.50
4	321.45	62.50	587.30	675.00	1130.45
5	224.75	114.60	313.85	380.50	1100.00
6	627.90	138.90	693.20	621.10	784.50
7	n.s.	68.20	266.65	n.s.	644.25

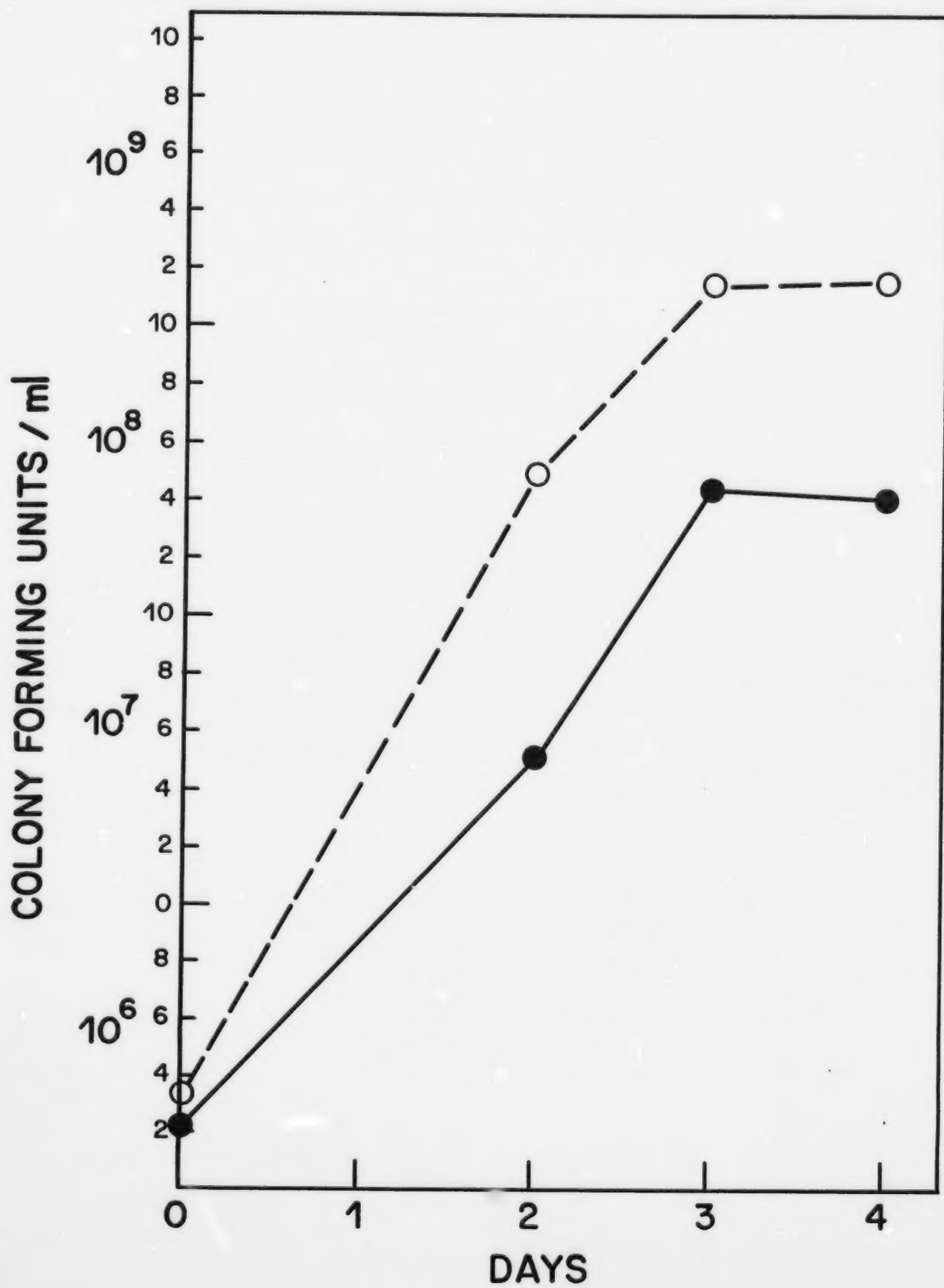


Figure 1

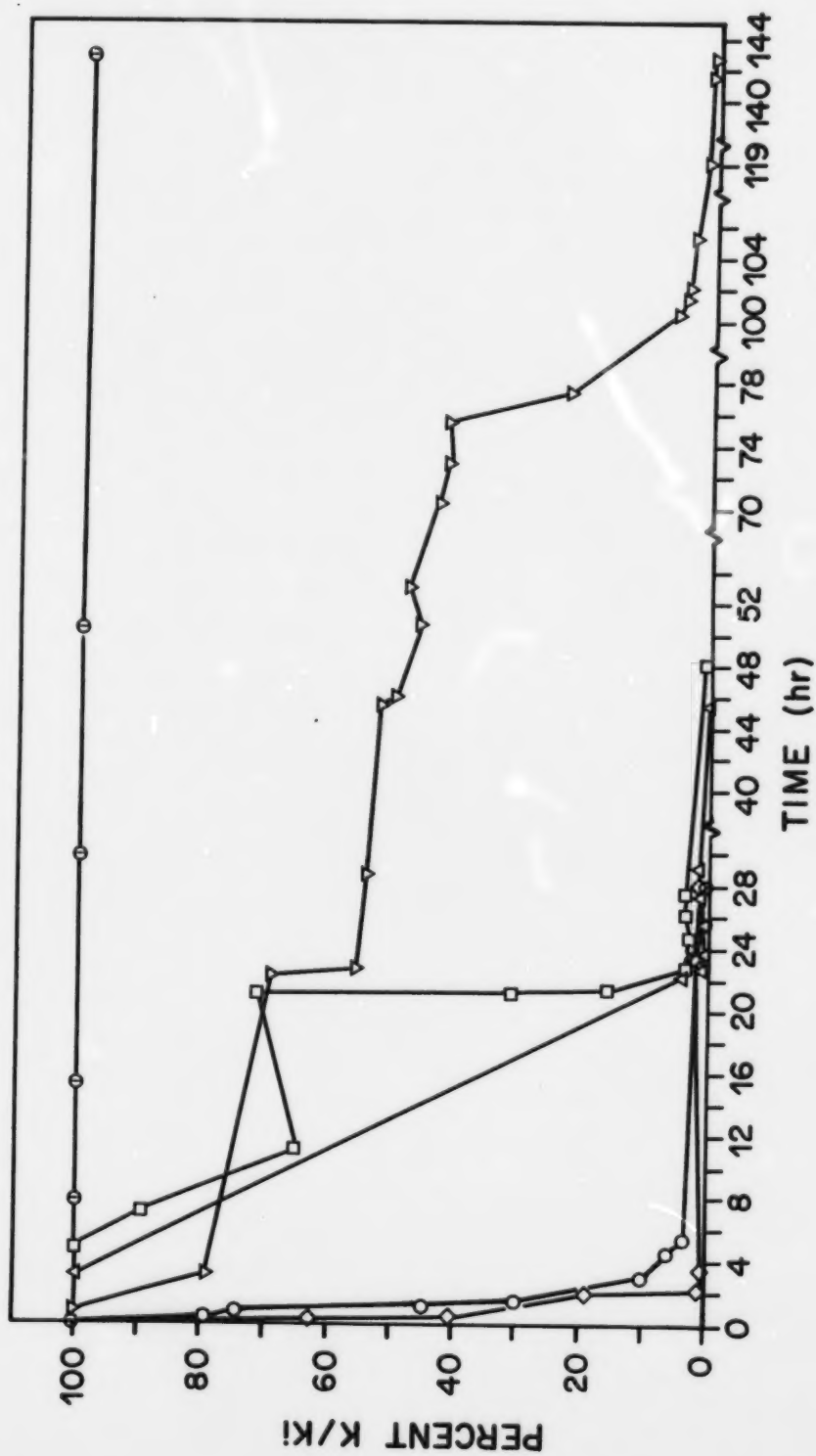


Figure 2

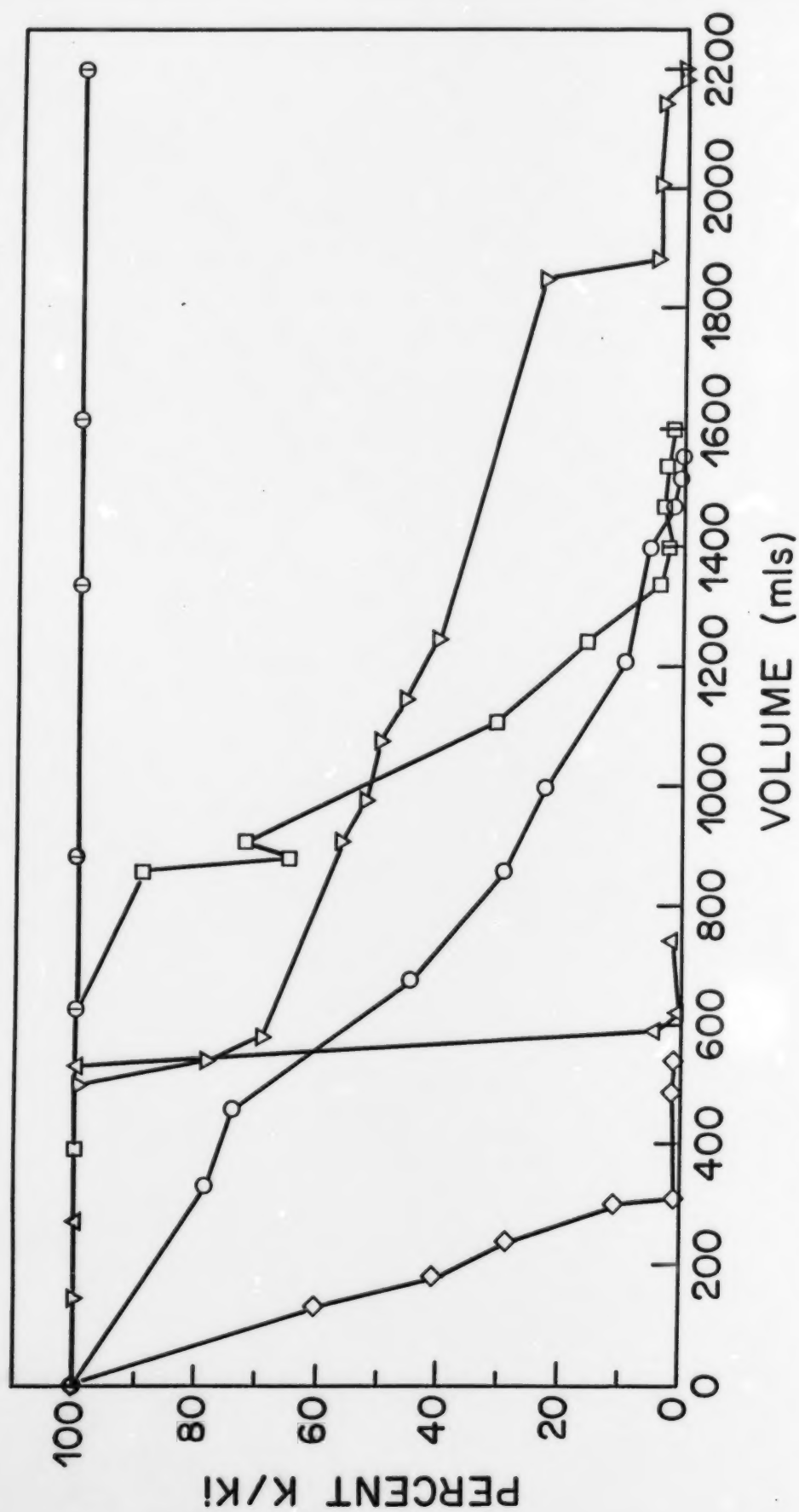


Figure 3

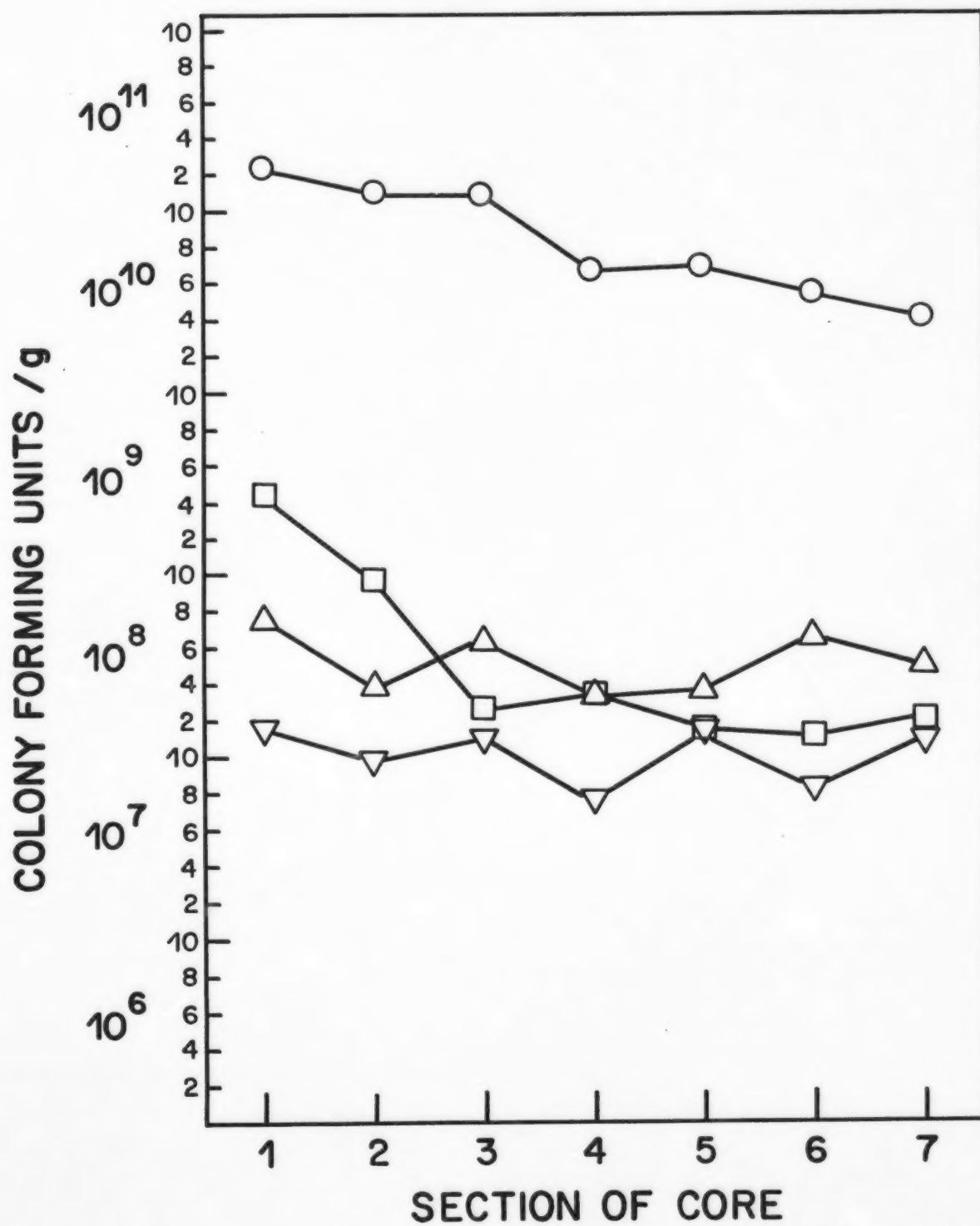


Figure 4

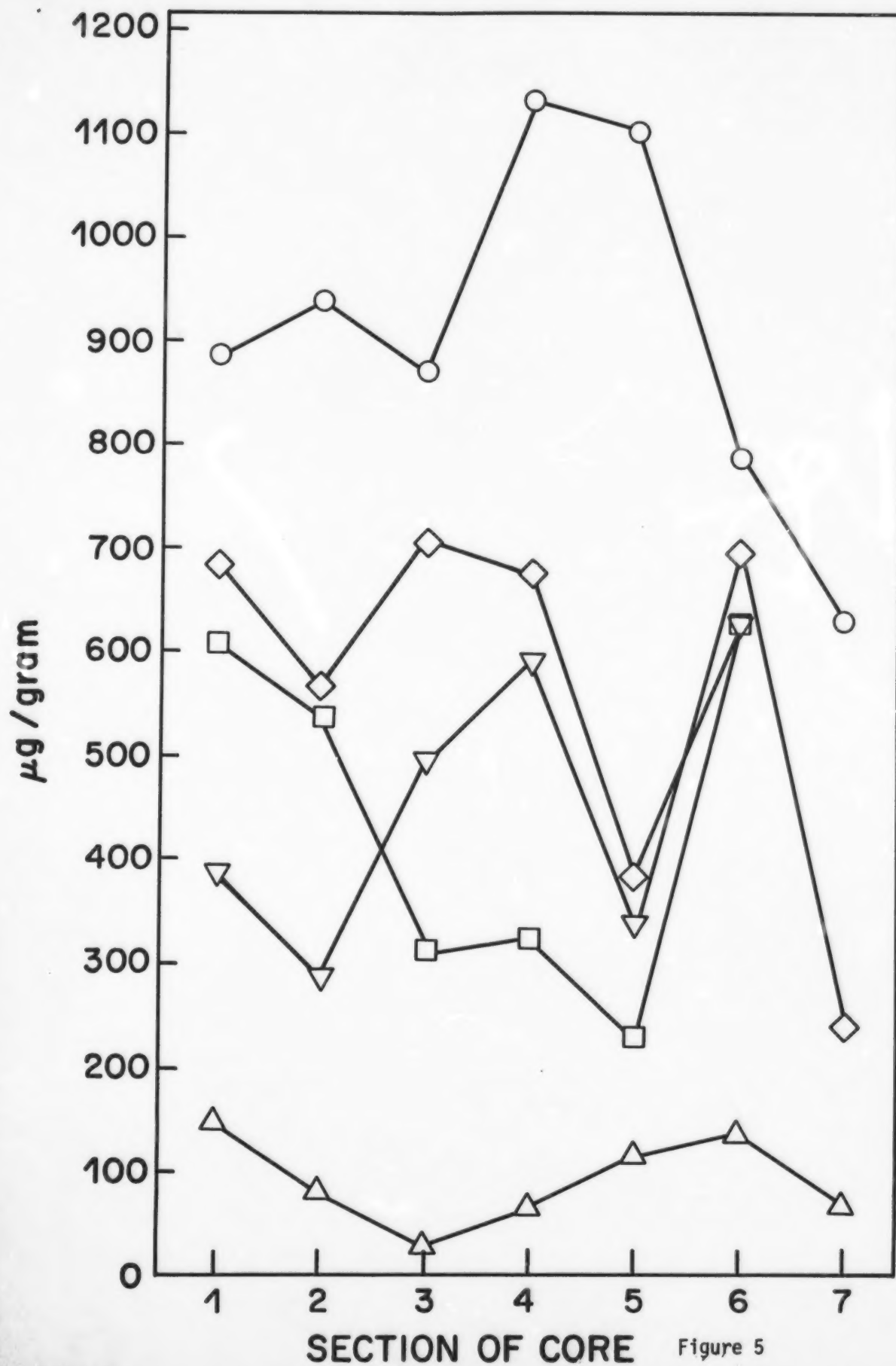


Figure 5



Figure b



Figure 7



Figure 8

APPENDIX 1

Chemically Defined Sporulating Medium (CDSM)

50 ml Macro Stock Solutions
10 ml Trace Element Solution
0.050 g l-Tryptophan
0.050 g l-Isoleucine

Volume to 500 ml with distilled water
Filter-sterilize

CDSM 10X Macro Stock Solution

20.9 g MOPS buffer (400 mM)
1.36 g KH_2PO_4 (40 mM)
3.14 g Ammonium sulfate (95 mM)
9.01 g Glucose (200 mM)
1.13 g l-Lactic acid (50 mM)
0.37 g l-Glutamic acid (10 mM)

pH to 7.0 with KOH
Volume to 250 ml with distilled water
Filter-sterilize

CDSM 50X Trace Element Solution

10.17 g Magnesium chloride
1.94 g Calcium chloride
0.25 g Manganese chloride
0.0004 g Zinc chloride
0.0203 g Ferric chloride
0.0169 g Thiamine HCl
0.5 ml of 1.0 N HCl

Volume to 250 ml with distilled water
Filter-sterilize

APPENDIX 2

Half-strength Brain Heart Infusion Medium (0.5 BHI)

18.5 g Difco BHI
1 L distilled water

Half-strength Brain Heart Infusion Agar (0.5 BHI)

18.5 g Difco BHI
20.0 g Difco Agar
1 L distilled water

Polymer Medium 2 (PM-2)

Equal mixtures of Base Medium (2x) and Phosphate Solution (2X)

PM-2 Base Medium (2X)

200 g Sodium chloride
0.5 g Magnesium sulfate
1.33 g Ammonium nitrate
2.0 g Sodium nitrate
1.0 g Yeast extract
2.6 g Citric acid
1 L distilled water

Adjusted to pH 8.0

PM-2 Phosphate Solution (2X)

10 g KH_2PO_4
1 L distilled water

Adjusted to pH 8.0

APPENDIX 3

Phosphate Buffered Saline (PBS)

8.7 g NaCl
1.23 g K_2HPO_4
0.4 g KH_2PO_4
1 L distilled water

Adjusted to pH 7.0

Sodium Citrate Medium (SCM)

6.45 g Citric acid trisodium salt
0.198 g $(NH_4)_2SO_4$
2.72 g KH_2PO_4
5.23 g K_2HPO_4
0.12 g $MgSO_4$
0.008 g $FeCl_2$
1 L distilled water

Adjusted to pH 7.0